Application No. 10/517,275
Filed on November 20, 2007
Response to Office Action dated May 21, 2007

AMENDMENTS TO SPECIFICATION:

Please replace the first full paragraph on page 27 with the following paragraph.

Example 2 - siRNA Synthesis and Transfection

The siRNA sequences were selected according to the method of Elbashir et al (23). The siRNA sequences specific for IL-12p35 (AACCUGCUGAAGGAUGGUGAC; SEQ ID NO:1), IL-12p40 (AAGAUG ACAUCACCUGGACCU; SEQ ID NO:2), and IFN-γ (AACTGGCAAAAGGATGGTGAC; SEQ ID NO:3) were synthesized and annealed by the manufacturer (Dharmacon Inc. Lafayette, CO). siRNA for IFN-γ was used as a control since bone marrow derived DC generated by the conditions described above did not produce IFN-γ after stimulation. Transfection efficiencies were determined using unlabeled and fluorescein labeled siRNA Luciferase GL2 Duplex (Dharmacon Inc). Transfection was carried out as described previously (Elbashir, S.M., 2002. Methods 26:199). Briefly, 3 μl of 20μM annealed siRNA was incubated with 3 μl of GenePorter (Gene Therapy Systems, San Diego, CA) in a volume of 100 μl RPMI-1640 (serum free) at room temperature for 30 min. This was then added to 400 μl of DC cell culture as described above. Mock controls were transfected with 3 μl GenePorter alone. After 4 hrs of incubation an equal volume of RPMI-1640 supplemented with 20% FCS was added to the cells. 24-48 hrs later, transfected DC were washed and used for subsequent experiments.

Please replace the paragraph bridging page 28, line 21 to page 29, line 2 with the following paragraph.

Example 5 - RT-PCR

Total RNA from siRNA-treated DC (10^6 cells) or from T cells purified from MLR (10^6 cells) was isolated by TRIzol reagent (Gibco BRL) according to the

manufacturer's instructions. First strand cDNA was synthesized using an RNA PCR kit (Gibco BRL) with the supplied oligo d(T)16 primer. One μmol of reverse transcription reaction product was used for the subsequent PCR reaction. The primers used for IL-12p35 and IL-12p40 flanked the sequences targeted by siRNA (IL-12p35, forward primer 5'-GCCAGGTGTCTTAGCCAGTC-3'~~,~~ [SEQ ID NO: 4]; reverse primer 5'-GCTCCCTCTTGTTGTGGAAG-3'~~,~~ [SEQ ID NO: 5]; IL-12p40, forward primer 5'-ATCGTTTTGCTGGTGT CTCC-3'~~,~~ [SEQ ID NO: 6]; reverse primer 5'-CTTTGTGGCAGGTGTACTGG-3' [SEQ ID NO: 7]). In addition, IL-10, IFN-γ, IL-4 and GAPDH (internal control) primers were used as previously described (Zhu, X., et. al., 1994. Transplantation 58:1104). The PCR conditions were: 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and PCR was done for 35 cycles. PCR products were visualized with ethidium bromide on 1.5% agarose gel.